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Description of *Trypanosoma* (*Megatrypanum*) *stefanskii* sp. n. from Roe Deer (*Capreolus capreolus*) in Poland[1]

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ABSTRACT: *Trypanosoma* (*Megatrypanum*) *stefanskii* sp. n. is described from 12 of 18 (66.6%) roe deer examined between 1984 and 1988 from Puszcza Niepolomicka in south-central Poland. Trypomastigotes were compared with unnamed trypanosomes from roe deer in Germany, with *T. cervi* Kingston and Morton, 1975, from North American cervids, with *T. theileri* Laveran, 1902, from North American cattle, and with *T. melophagium* Flu, 1908, from sheep in Germany. Qualitatively, trypanosomes from roe deer in Poland differed from all the above in that 90 (56%) of 162 specimens examined lacked a free flagellum extending beyond the body. Trypanosomes from roe deer in Poland differed also in most mensural values from trypanosomes from roe deer in Germany and from trypanosomes from North American cervids and cattle. Trypanosomes from roe deer in Poland most closely resemble *T. melophagium* from sheep in Germany, but conspecificity is not considered possible inasmuch as the latter species is held to be markedly host specific. A discussion of *Trypanosoma* (*Megatrypanum*) spp. from various hosts in Europe and North America is provided.

KEY WORDS: *Trypanosoma stefanskii* sp. n., (*Megatrypanum*), roe deer, *Capreolus capreolus,* Poland.

Ruminant stercorarian trypanosomes, subgenus *Megatrypanum,* were known, until recently, from only a limited number of host species worldwide. They included *Trypanosoma theileri* Laveran, 1902, from bovids (principally cattle, *Bos taurus* L., 1766); *T. melophagium* Flu, 1908, from sheep, *Ovis aries* L.; and *T. theodori* Hoare, 1931, from goats, *Capra hircus* L. Wrublewski (1909, 1912) reported *T. theileri* from European bison, *Bison bonasus* L., from Puszcza Bialowieska in eastern Poland although some controversy arose regarding this designation (Wladimiroff and Yakimoff, 1909; Yakimoff, 1915).

Reports of trypanosomes from cervids were also rare. Knuth (1909) reported on a species of "*Herpetomonas*" (surely an erroneous identification) observed in blood films from the heart of a roe deer, *Capreolus capreolus* Gray, 1821, from Westerwald district in Germany. *Trypanosoma evansi* Balbiani, 1888, a salivarian trypanosome, was reported from *Cervus unicolor* (Kerr, 1792) in Mauritius (Adams and Lionnet, 1933); in muntjak, *Muntiacus muntjak* Zimmermann, 1780, the axis deer, *Axis axis* Erxleben, 1777, and *Cervus timorensis* (Blainville, 1822) in Indonesia (Kraneveld and Mansjoer, 1952); and from roe deer in the Soviet Union (Kazakhstan) (Galuzo and Novinskaia, 1958). In the Western Hemisphere, stercorarian, subgenus *Megatrypanum,* trypanosomes, *T. mazamarum* Mazza, Romana, and Fiora, 1932, and *T. theileri*-like forms were recovered from *Mazama* spp. Rafinesque, 1817, and *Odocoileus virginianus* (Zimmermann, 1780) in Argentina and Brazil (Mazza et al., 1932; Deane, 1961) and Colombia (Ayala et al., 1973). In North America the first report of a *Trypanosoma* sp. in cervids (white-tailed deer, *Odocoileus virginianus*) was from blood cultured from deer from the southeastern United States (Kistner and Hanson, 1969). Subsequently, *Trypanosoma* sp. was reported from mule deer (*Odocoileus hemionus* (Rafinesque, 1817)) in Colorado and New Mexico (Clark, 1972) and Wyoming (Kingston et al., 1975); elk (*Cervus canadensis* (Erxleben, 1779)) in Wyoming (Kingston and Morton, 1973) and Colorado and New Mexico (Davies and Clark, 1974); reindeer (*Rangifer tarandus* L.) in Alaska (Kingston et al., 1982) and Finland (Kingston and Nikander, 1985); and moose (*Alces alces* L.) in Alaska and Wyoming (Kingston et al., 1981).

More recently, *Trypanosoma* (*Megatrypanum*) spp. were reported from red deer (*Cervus elaphus* L.), fallow deer (*Cervus dama* L.), and roe deer from Germany (Hoffman et al., 1984),

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Table 1. Morphological comparison of trypanosomes from roe deer, *Capreolus capreolus*, from Poland with trypanosomes from cervid species and cattle from North America and with trypanosomes from roe deer and sheep from Germany.

Host species	PK	KN	PN	NA
Roe deer (84) *Trypanosoma stefanskii* Poland $N = 50$	15.9 ± 6.39* 0–28†	5.8 ± 2.07 0–10	23.3 ± 5.59 13–33	32.0 ± 5.1 20–40
Roe deer (88) *Trypanosoma stefanskii* Poland $N = 40$	14.2 ± 5.66 5–27	5.8 ± 1.32 3–9	19.88 ± 6.3 9–33	28.2 ± 6.07 14–39
Roe deer (88) *Trypanosoma stefanskii* Poland $N = 72$	13.5 ± 5.32 2–24	6.3 ± 1.96 3–15	19.57 ± 4.94 11–30	27.8 ± 6.71 11–42
Composite roe deer *Trypanosoma stefanskii* Poland $N = 162$	14.39 ± 5.81 0–28	6.0 ± 1.86 0–15	20.8 ± 5.73 9–33	29.2 ± 6.36 11–42
Roe deer‡§ *Trypanosoma* sp. Germany $N = 86$	9.8 ± 5.7	5.8 ± 1.4	15.5 ± 5.7	18.1 ± 5.0
All deer‖ *Trypanosoma cervi* North America $N = 174$	11.5 ± 5.60 3–27	7.0 ± 2.10 2–14	18.5 ± 6.34 8–36	23.3 ± 7.30 10–43
Cattle¶ *Trypanosoma theileri* North America $N = 304$	7.4 ± 3.3 0–17	8.9 ± 2.6 2–20	16.2 ± 5.1 5–33	20.2 ± 6.3 7–36
Sheep**§ *Trypanosoma melophagium* Germany $N = 111$	14.7 ± 2.9	5.1 ± 1.1	19.8 ± 3.5	19.5 ± 1.9

* SD.
† Range.
‡ Hoffman et al., 1984 (roe deer).
§ Range not given.
‖ Includes elk, Kingston and Morton, 1975; mule deer, Matthews et al., 1977; white-tailed deer, Kingston and Crum, 1977; reindeer, Kingston et al., 1982; moose, Kingston et al., 1985.

and in roe deer (Kingston and Bobek, 1985), red deer, and elk in Poland (Kingston et al., 1985a). Hinaidy (1987) reported on the recovery of *Trypanosoma* (*Megatrypanum*) sp. from 2 of 105 roe deer in Austria. None of the European reports identified trypanosomes from deer as other than *Trypanosoma* (*Megatrypanum*) spp. Also, *Trypanosoma* (*Megatrypanum*) sp. was rediscovered in 4 of 38 wisent, i.e., European bison, *Bison bonasus*, in Poland from Puszcza Bialowieska (Kingston et al., 1987).

Kingston and Morton (1975) compared hemoflagellates from cattle and elk in North America and concluded that the form from elk was a new species, which they designated *Trypanosoma* (*Megatrypanum*) *cervi* Kingston and Morton, 1975. Subsequently, trypanosomes from mule deer (Matthews et al., 1977), white-tailed deer (Kingston and Crum, 1977), reindeer (Kingston et al., 1982), and moose (Kingston et al., 1985b) were considered as conspecific with *T. cervi* from North American elk. Cross-transmission experiments of *T. cervi* from elk to cattle (6 trials—intact and splenectomized recipients) failed to produce infections in the experimental, putative recipient hosts (Kingston and Morton, 1973). Blood containing *T. cervi* from an infected mule deer was transferred to an uninfected elk in which

Table 1. Continued.

BL	FF	L	W	FF:BL	KI	NI
55.1 ± 9.23 37–71	0	55.1 ± 9.23 34–71	5.66 ± 1.65 3–10	00	1:3.66 ± 1.44 0–8	0.73 ± 0.15 0.43–1.1
48 ± 11.5 26–70	0	48 ± 11.5 26–70	5.8 ± 1.94 2–11	00	1:3.46 ± 0.89 1.7–5.4	0.71 ± 0.15 0.41–0.93
47.3 ± 10.3 26–68	7.7 ± 2.73 4–17	55 ± 10.34 37–75	6.53 ± 2.5 2–13	1:6.9 ± 2.72 1:2–13.4	1:3.34 ± 1.09 1–6	0.73 ± 0.25 0.41–2.27
49.9 ± 10.81 26–71	7.7 ± 2.73 0–17	55.02 ± 10.34 26–75	6.1 ± 2.16 2–13	1:6.9 ± 2.72 1:2–13	1:3.47 ± 1.17 0–8	0.73 ± 0.20 0.41–2.27
33.6 ± 9.5	10.4 ± 2.5	44.0 ± 8.5	not given	1:2.93 ± 7.72	1:2.9 ± 0.98	0.87 ± 0.16
42.0 ± 12.44 21–74	8.2 ± 3.24 0–21	50.1 ± 13.64 26–83	5.5 ± 2.48 1–13	1:6.1 ± 3.24 1:0–27	1:2.7 ± 0.96 1:1.23–7	0.8 ± 0.22 0.42–2.67
36.4 ± 10.5 13–59	14.2 ± 4.5 1–37	50.5 ± 12.7 16–90	3.3 ± 2.02 1–13	1:2.8 ± 2.29 1:0.89–39	1:1.86 ± 0.42 1–4	0.88 ± 0.22 0.43–1.67
39.3‡‡	6.0 ± 1.6	45.3 ± 4.1	3.1‡‡	1:6.55‡‡	1:3.8‡‡	1.1‡‡

¶ Matthews et al., 1979; McKenzie, unpublished M.S. thesis, 1980.
** Buscher and Friedhoff, 1984.
‡‡ SD not given.
Underlined data calculated.

a cryptic infection (detected by culture only) resulted, persisting for approximately 30 days (Matthews et al., 1977). In a third experiment, *T. cervi* from an infected reindeer from Alaska were inoculated into 2 uninfected elk calves and an uninfected bovine calf but failed to result in infection (Kingston et al., 1982). Living, culture derived, cryopreserved trypanosomes from cattle (*T. theileri*) and North American bison (*Trypanosoma* sp.) were inoculated into homologous and heterologous hosts. A transient infection developed in 1 bison inoculated with *T. theileri*; no infections developed in the other recipients. The trypanosomes recovered from the experimentally infected bison were compared with *T. theileri* from Wyoming cattle (Matthews et al., 1979) and the forms from these hosts were considered conspecific (Kingston et al., 1986). Recent transmission studies in Germany using naturally infected species of tabanid flies harboring species of cattle and deer trypanosomes have also demonstrated that trypanosomatid species from cattle and deer are distinct (Bose et al., 1987).

Although trypanosomes from a relatively large number of cervid (and other) hosts from North and South America, Germany, Austria, and Poland have been described and some named, many

remain nameless pending further analysis. The description, comparison, and analysis of 162 trypanosomes of the new species from 12 of 18 roe deer collected in Puszcza Niepolomicka in south-central Poland in 1984 and 1988 form the basis for this paper. The species is named in honor of the late Prof. dr hab. Witold Stefanski, founder of the Institute of Parasitology-PAN, which bears his name.

Materials and Methods

Blood samples were collected in heparinized or plain tubes from the heart, pleural, or body cavities of hunter-killed male roe deer (*Capreolus capreolus*) during 1–10 August 1984 (8 deer) and 22–31 July 1988 (10 deer) in Puszcza Niepolomicka (south-central Poland). Direct examinations (DE) of samples using phase, or brightfield, microscopy were conducted within 2 hr following collection using the microhematocrit concentration technique of Bennett (1962). When trypanosomes were detected microscopically (10× objective) in tubes swimming in plasma, or serum, above the buffy coat, tubes were scored, broken, and trypanosomes, plasma or serum, white blood cells, and a few red blood cells, to serve as a marker, were expressed onto microscope slides using a stylet to push against the sealant (Crito seal®). Conventional thin blood films were prepared, air-dried, fixed in absolute methanol, and stained in Giemsa's stain, and later examined microscopically. Trypanosomes were photographed using a photo-equipped Reichert Zetopan compound microscope at 1,000× on color slide film (Orwochrome or Kodachrome 25). A stage micrometer was also photographed at the same magnification to allow for calibration of the measuring device (Curvometer, Alvin 1112). The processed slides were projected from a standard distance and the trypanosome images sketched onto tracing paper. The sketches were measured (Hoare, 1972; Kingston and Morton, 1975) for various morphological parameters: PK = posterior end to kinetoplast, KN = kinetoplast to nucleus, PN = posterior end to nucleus, NA = nucleus to anterior end, BL = body length, FF = free flagellum, L = length, W = width; and various indices calculated, FF:BL = free flagellum to body length ratio, NI = PN/NA, KI = PN/KN. Results were recorded in μm after calculating values from the projected image of the stage micrometer and were processed through a VAX8800 computer, using SPSS-X Data Analysis System 3.0 (Statistical Package for the Social Sciences, Version 8) for a one-way analysis of variance utilizing Duncan's multiple range test to compare the differences (confidence interval, $P \leq 0.05$) between group means of trypanosomes from North American deer and cattle, and roe deer from Poland.

Results

Mensural values of *T. stefanskii* from roe deer from Poland were compared to similar values of *T. cervi* from North American deer and *T. theileri* from cattle (Table 1).

Seven of 8 roe deer from Puszcza Niepolomicka sampled in early August 1984 were infected with trypanosomes (Kingston and Bobek, 1985). Trypanosomes from 4 of the deer were studied ($N = 50$) and all apparently lacked a free flagellum. Most specimens were trypomastigotes, but some broad, predivision forms with 2 kinetoplasts and sometimes with a short flagellum were seen. Further sampling in late July 1988 in the same locality resulted in recovery of trypanosomes ($N = 426$ trypanosomes on 71 slides) from 5 of 10 bucks examined. Seventy-two of 112 trypanosomes studied possessed a free flagellum 7.7 (4–17) μm in length (Fig. 1); the remaining 40 lacked a free flagellum (Fig. 2). Some specimens on a given slide lacked a free flagellum, whereas other specimens on the same slide possessed one. In both types of trypanosome, the undulating membrane was usually well developed. With the exception of this flagellar difference, a one-way analysis of variance (ANOVA) of the means of other mensural values revealed no significant differences ($P \leq 0.05$). Some differences ($P \geq 0.05$) were noted in PN, NA, and BL of trypanosomes from deer sampled in 1984 and those examined in 1988. It was not possible to compare FF:BL ratios where FF was lacking.

All 112 trypanosomes examined in 1988 were from 3 of the 5 infected deer. No differences were noted in PK, KN, FF (where present), W, NI, and KI ($P \leq 0.05$). Some variation ($P \geq 0.05$) was seen in specimens from 2 of the deer in PN, AN, BL, L, and the FF:BL ratio. These variations are essentially reflections of differences in body length.

Discussion

Trypanosoma cervi from North American deer, and *T. stefanskii* from roe deer in Poland, superficially appear to be similar. Nearly all mensural values (PK, KN, PN, NA, BL, W, KI, and FF:BL), however, were found to be significantly different. Total length values for *T. theileri* and *T. cervi* are similar ($P \leq 0.05$); however, this similarity is a reflection of the shorter FF of *T. cervi* (8 μm) as compared with *T. theileri* (14 μm). Free flagellar lengths of *T. cervi,* and of *T. stefanskii* (which possessed this organelle), did not differ significantly ($P \leq 0.05$), 8.2 and 7.7 μm, respectively. Nuclear index (NI) values for *T. theileri* and *T. cervi* did not differ significantly ($P \leq 0.05$), whereas this value for *T. stefanskii* from roe deer differed from both ($P \geq 0.05$) (Table 1).

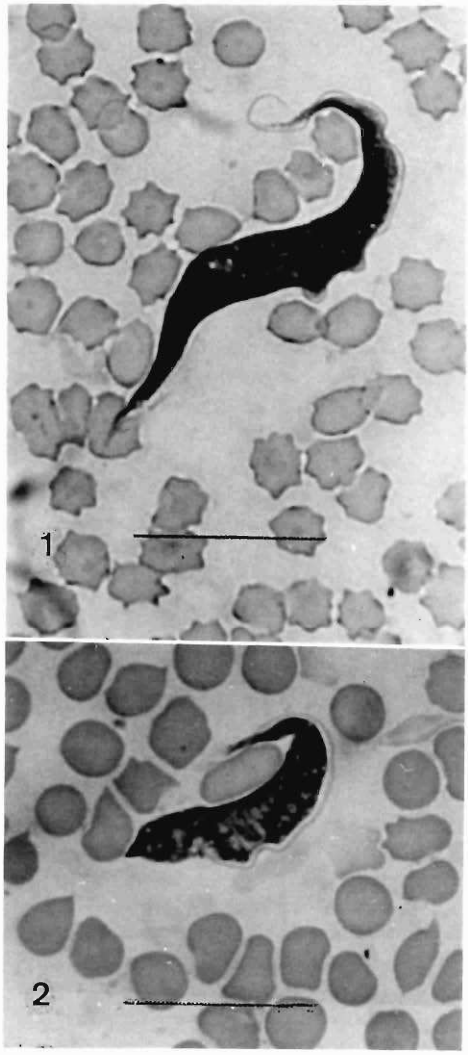
Except in mean NA, BL, and W values, *T.*

stefanskii from roe deer in Poland closely resembles *Trypanosoma melophagium* from sheep in Germany (Table 1) (Buscher and Friedhoff, 1984). This resemblance must be considered superficial if one adheres to the widely held principle of strict host specificity of most stercorarian trypanosomes (Hoare, 1972).

Trypanosomes from roe deer in Poland collected in 1984 and 1988 differed from trypanosomes reported from roe deer in Germany collected in 1983 (Hoffman et al., 1984) (Table 1). Qualitatively, 90 of 162 (55.6%) of the trypanosomes from roe deer in Poland lacked a free flagellum, whereas all 86 of the trypanosomes from Germany possessed this organelle (Hoffman et al., 1984). The basis for this difference is unknown. These aflagellate forms from Polish roe deer may represent the vector-infective, aflagellate, so-called "stumpy" forms seen in other species of trypanosomes (Hoare, 1972), but never noted by us in North American *Megatrypanum* species. Also, the mean FF (when present in the Polish material) values between these 2 groups of trypanosomes differed, those from Germany being approximately 25% longer (10.4 vs. 7.7 μm, respectively). Although unclear, this anomalous condition may have resulted from genetic drift producing forms with and without a free flagellum in the same parasite species. Other quantitative differences are obvious in PK, KN, NA, BL, and possibly in L, FF:BL, and KI values, all being smaller in the German material. The only numerical correspondence appears to occur between trypanosomes from roe deer from Poland which lack a free flagellum and those from trypanosomes from roe deer in Germany (all of which had a free flagellum) in KN values.

Based on morphologic data, there appear to be 2 or more distinct populations of *Megatrypanum* trypanosomes in Europe. In the West, trypanosomes appear homogeneous in the possession of a free flagellum and otherwise quantitatively different from forms found more easterly which appear homogeneous in all values except for the presence or absence of a free flagellum. Hoffman et al. (1984) implied that the trypanosome from roe deer was, perhaps, a separate species but felt cross-transmission experiments were needed to satisfy this question.

The trypanosomal material from roe deer in Poland is clearly morphologically distinct from trypanosomes from North American deer spp. (except for FF values), perhaps owing to host differences or their long-term temporal and wide geographic separation. In addition, these trypanosomes differ from trypanosomes in Germany. Thus, the trypanosomes from roe deer in Poland warrant specific recognition and are designated *Trypanosoma stefanskii* sp. n.



Figures 1, 2. ***Trypanosoma (Megatrypanum) stefanskii* sp. n. 1. Bloodstream trypomastigote with free flagellum, roe deer. 2. Bloodstream trypomastigote lacking free flagellum, roe deer. Scale bar = 20 μm.**

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1991 Honor Award

Helminthological Society of Washington member Isi A. Siddiqui, Assistant Director of Food and Agriculture, received the 1991 Honor Award from NASDA. His accomplishments, for which the award was given, were in pest prevention in California, particularly Mexican and Mediterranean fruit fly eradication programs.

Dr. Isi A. Siddiqui (right) receiving the 1991 Honor Award from the National Association of State Departments of Agriculture.